VOLUME 10 ISSUE 1 2024

ISSN 2454 – 3055

INTERNATIONAL JOURNAL OF ZOOLOGICAL INVESTIGATIONS

Forum for Biological and Environmental Sciences

Published by Saran Publications, India

International Journal of Zoological Investigations Vol. 10, No. 1, 1017-1034 (2024)

International Journal of Zoological Investigations

Contents available at Journals Home Page: www.ijzi.net

Editor-in-Chief: Prof. Ajai Kumar Srivastav

Published by: Saran Publications, Gorakhpur, India

ISSN: 2454-3055

Targeting Lymph Nodes with a Nanoparticle-Based Drug Delivery System

Loushambam Samananda Singh, Peter De Roux Sumer, Waibiangki Lyngdoh, Akash Sharma, Phaibiang Lapasam and Bimal Debbarma*

Institute of Pharmacy, Assam Don Bosco University, Tapesia, Assam 782402, India

**Corresponding Author*

Received: 24th November, 2023; **Accepted:** 22nd January, 2024; **Published online:** 25th June, 2024

https://doi.org/10.33745/ijzi.2024.v10i01.106

Abstract: The lymphatic system is essential for moving substances from tissues in the body's outer regions to lymph nodes, producing immune responses before circulating throughout the body. Numerous lymph nodes, which also serve as sites for lymphocyte accumulation, activation, and proliferation, house a significant portion of the body's immune cells. The ability to deliver drugs directly to lymphocytes and cells residing in lymph nodes offers the chance to modify the adaptive immune response. The lymphatic system can be effectively targeted to greatly aid in the treatment of disease and boost the efficacy of vaccination. Recently, interest has increased in creating drug delivery systems that specifically target lymph nodes using nanotechnologies and novel biomaterials. Nanoparticles have several benefits that make effective lymphatic system delivery possible. The lymphatics offer a promising pathway for delivering immune-modulatory therapies to lymph nodes, where immunotherapies can be improved, and they also function as an alternative route to general circulation. In this review, we concentrate on materials moving from peripheral tissues to lymph nodes and nanomaterial-based delivery strategies that make use of the potential of lymphatic transport to either deliver therapeutics to lymph nodes or the bloodstream.

Keywords: Lymphatic system, Lymph nodes, Nanoparticles, Drug-delivery system, Immunity

Citation: Loushambam Samananda Singh, Peter De Roux Sumer, Waibiangki Lyngdoh, Akash Sharma, Phaibiang Lapasam and Bimal Debbarma: Targeting lymph nodes with a nanoparticle-based drug delivery system. Intern. J. Zool. Invest. 10(1): 1017-1034, 2024.

https://doi.org/10.33745/ijzi.2024.v10i01.106

Introduction

The lymphatic system's primary function is to remove extra interstitial fluid from the tissues and return it to the bloodstream. The lymphatic vessels have one-way valves that allow the lymphatic fluid to flow toward the thoracic cavity, where it is eventually returned to the bloodstream via the thoracic duct. This process helps to prevent fluid accumulation in the tissues, leading to swelling or edema (Cote *et al.*, 2019). The lymphatic system also plays a role in immune function, as it helps transport immune cells and other substances throughout the body to fight off infections and other foreign invaders (Liao and von der Weid, 2015). The lymphatic system

includes lymphatic vessels, lymph nodes (LNs), the spleen, the thymus, and other lymphoid tissues. These structures play a crucial role in the body's immune response by providing a site for antigen presentation and immune activation (Trevaskis *et al.*, 2015). The lymphatic system serves a vital function in transporting a variety of substances, including fluids, macromolecules, particles, and cells, from peripheral tissues to the bloodstream. This includes transporting antigens, antigen-presenting cells (APCs), and lymphocytes involved in the immune response (Randolph and Miller, 2014).

LNs are specialized structures that are distributed throughout the lymphatic system, as lymph flows from the lymphatic capillaries towards the subclavian veins, it passes through several LNs, which are densely populated with immune cells such as macrophages, dendritic cells, B cells, and T cells (Willard-Mack, 2006). Subcapsular sinus (SCS) macrophages and dendritic cells (DCs) are two important types of resident immune cells found in LNs. SCS macrophages are in the subcapsular sinus, which is the outermost region of the lymph node, and they are responsible for filtering lymph and capturing antigens. DCs, on the other hand, are in the deeper regions of the lymph node, such as the T-cell zone and the follicles, and they are responsible for presenting antigens to T cells and B cells. When antigens are captured by SCS macrophages, they are transported to the DCs in the deeper regions of the lymph node, where they are presented to naive T cells and B cells. This process activates the adaptive immune response, which produces specific antibodies and T cells that can recognize and eliminate the antigen (Jarjour *et al.*, 2014).

B cells and T cells, the two major types of lymphocytes involved in adaptive immune responses, use various cell surfaces proteins such as C-C chemokine receptor 7 (CCR7), lymphocyte function-associated antigen 1 (LFA-1), and L-selectin to navigate through the high endothelial venules (HEVs) that lead into the LNs. Once inside the LNs, B cells, and T cells undergo clonal expansion and differentiation in their respective zones, which are organized to optimize interactions between APCs and lymphocytes (Ruddle, 2016). LNs are of the utmost importance in controlling immune responses, and this has led to a great deal of research into how to develop immunotherapeutic approaches for a variety of diseases, including cancer and autoimmune diseases. For instance, some immunotherapies aim to stimulate or enhance immune responses by targeting specific immune cells or molecules within LNs, while others aim to modulate or dampen immune responses to prevent autoimmunity or transplant rejection (Spurrell and Lockley, 2014; Wennhold *et al.*, 2019).

The LNs can be an important target for chemotherapeutic drug delivery, especially in the treatment of lymphomas and other cancers that have spread to the LNs. Chemotherapeutic drugs can be delivered to LNs using various methods, such as intravenous injection, lymphatic mapping, and intra-nodal injection. In some cases, drug delivery can be enhanced by using nanoparticles or liposomes that can specifically target lymphatic vessels or immune cells within the LNs (Ulintz *et al.*, 2018; Wang *et al.*, 2018). In addition, LNs, like lymphatic vessels, have a smooth muscle layer that allows them to contract and pump lymphatic fluid through the lymphatic system. This movement of lymphatic fluid can help to distribute drugs throughout the lymphatic system and to LNs (Margaris and Black, 2012). However, it can also make drug delivery to specific LNs challenging, as the movement of lymphatic fluid can rapidly clear drugs from the lymphatic system. In drug delivery, understanding the role of the lymphatic system in disease can also lead to the development of new diagnostic and prognostic tools. Modifications in lymphatic function or variations in the size and structure of LNs can be used to identify and track the progression of several diseases, including cancer and inflammatory conditions.

Drug efficacy depends on how much of the drug reaches the target site and how long it stays

there. Side effects and toxicity can limit the amount of drug that can be given. Biomaterials can help by prolonging circulation time or improving tissue targeting, allowing for specific cells to be targeted. Carriers like polymers, lipids, and inorganic materials can change how the drug behaves in the body. Different materials are being studied for delivering drugs to LNs, including micelles, dendrimers, nanoparticles, and liposomes. These materials improve lymph node targeting by increasing drug weight, reducing blood vessel permeability, targeting immune cells in peripheral tissues, or using a combination of these methods. Each material has unique advantages for specific applications and targets (Schudel *et al.*, 2019). This review focuses on the use of materials that target specific cells within LNs, which are important targets for immunotherapy and targeted drug delivery. It looks into how molecules and cells move to and within LNs and discusses how bioinspired materials can be used in immunology research and drug delivery.

LNs' Important Role in Physiological Processes:

The lymph node is composed of numerous lymphoid lobules, which are the fundamental functional and structural components of the node. These lobules cover the SCS and are surrounded by a protective capsule. The activation and differentiation of T cells and B cells into effector cells in adaptive immunity are enabled by the gathering of antigens, APCs, and immature lymphocytes in LNs. This concentration allows for effective interaction and processing, resulting in a heightened immune response to the specific antigen (Jalkanen and Salmi, 2020a). Most leukocytes, including activated DCs, effector and memory lymphocytes, and monocytes, can move into the afferent lymphatic vessels within the adjacent tissues. This enables their transportation to the closest lymph node, where they can contribute to immune reactions (Moran *et al.*, 2019). Antigens and other soluble molecules present in the interstitial fluid can enter the afferent lymphatic vessels. These cells and molecules are subsequently carried by the lymph fluid to the draining LNs through a one-way flow. Moreover, the macrophages and DCs that are associated with the sinus in the subcapsular region of the LNs are arranged within or beneath the Lymphatic endothelial cells (LECs) in this area (Gerner *et al.*, 2015). The macrophages and DCs are situated inside the subcapsular sinus. In the cortical and accessory cortical regions of the LNs, there is a network of reticular cells and fibroblasts that are distributed throughout and are referred to as the conduit system (Moran *et al.*, 2019). The countercurrent exchange of lymphocytes and APCs within lymphoid tissues allows for efficient recognition and selection of antigen-specific lymphocytes. As lymphocytes and APCs flow through the lymphoid tissue, they encounter each other and engage in a series of interactions that can lead to the activation and proliferation of specific lymphocyte clones. The countercurrent exchange helps to increase the likelihood of these interactions occurring by bringing together a larger number of lymphocytes and APCs (Roozendaal *et al.*, 2008). LNs that are small in size have a minimal number of lobules, sometimes just one. The afferent lymphatic vessels connect to the LNs on one side and are compartmentalized into the cortex, accessory cortex, and medulla, which then connect with the efferent lymphatic vessels. At the periphery of the lymph node, the SCS links directly to the medullary sinus (Willard-Mack, 2006). The afferent lymphatic vessels have sinuses and efferent lymphatic vessels that consist of a uniform layer of LECs. However, the characteristics of these LECs can differ depending on their specific location (Jalkanen and Salmi, 2020b). Lymphatic drainage has effects beyond reducing tissue swelling. It facilitates the movement of both external and self-generated antigens to the LNs, which helps regulate immune responses, including the activity of regulatory T cells and immune tolerance (Thomas *et al.*, 2012). Additionally, it can locally suppress the body's natural defenses against tumors, and play a role in the reconstruction of the LNs involved in draining fluids from the affected area (Mebius *et al.*, 1991;

Lund *et al.*, 2012). The lymphatic system's ability to circulate lymph fluid and transport immune cells throughout the body is closely linked to its function in immunophysiology. Efficient lymphatic vessel transport is essential for optimal immune response and overall health, and any problems with this transport system can have negative consequences (Thomas *et al.*, 2016).

Lymph is formed when fluids, immune cells, macromolecules, and molecules are enclosed in lipoproteins, vesicles, or exosomes and enter the initial lymphatic vessels. The lymph then flows through a network of enlarged collecting vessels, LNs, and post-nodal vessels before converging on the left or right thoracic lymphatic vessels. Finally, the lymph is emptied into the venous system. Therapeutic drugs can be targeted to the lymphatic system via mucosal, intestinal, or parenteral routes. Particulate matter transported through the mucosal pathway is absorbed by the mucosa-associated lymphoid tissue through the epithelium. Intestinal or oral fatty drugs are incorporated into intestinal lipoprotein collections and transported to intestinal lymphatic vessels. Finally, macromolecules transported through the parenteral or interstitial pathway enter the lymphatic capillaries because they are too large to enter the capillaries at the injection site for drainage (Trevaskis *et al.*, 2008, 2015; Yáñez *et al.*, 2011). Dysfunction in the lymphatic system can contribute to the development and progression of various diseases such as lymphedema, immune and inflammatory diseases, cancer and tumor metastasis, and metabolic diseases. As researchers have come to realize the wide-ranging roles of lymphatic vessels in disease, it has become increasingly important to comprehend the diverse functions of the lymphatic system to identify potential therapeutic targets for these conditions (Proulx *et al.*, 2013; Dieterich *et al.*, 2014). LNs act as a vital link between innate immunity and acquired immunity. In several diseases, there may be variations in lymphatic angiogenesis, lymphatic vessel density, expansion, constriction, and lymphatic flow. However, the significance of these modifications in terms of their function remains uncertain (Trevaskis *et al.*, 2015).

The dissemination of disease through the lymphatic system is a prevalent mode of transmission for cancer and other illnesses. This factor is closely associated with cancer mortality rates and represents a critical challenge for cancer treatment and management (Kato *et al.*, 2020). Cancer cells, bacteria, and viruses can disseminate through lymphatic vessels, enter the bloodstream, and establish secondary tumors or sites of infection (Kato *et al.*, 2019). Typically, the primary tumor infiltrates the nearby draining LNs, serving as a reservoir for the further spread and metastasis of cancerous cells (Tseng *et al.*, 2014). Cancer cells that have spread to the LNs can infiltrate the blood vessels within the LNs even before the neighbouring LNs are affected, thus initiating systemic metastasis (Takeda *et al.*, 2017). This implies that metastatic LNs can serve as an origin for systemic metastasis. In various types of cancers, such as breast cancer, head and neck squamous cell carcinoma, and lung cancer the predominant mechanism for tumor cell transfer to LNs is through tumor-associated lymphatic vessels during metastatic progression from the primary tumor (Swartz and Lund, 2012; Karaman and Detmar, 2014).

When a tumor forms and grows, an excess of angiogenic signals can cause irregular changes in the tissue structure, resulting in the stretching, enlargement, and breakage of the tumor's blood vessels, which can also trigger the growth of lymphatic vessels (Rohner and Thomas, 2016). The lymphatic vessels surrounding a tumor are more concentrated than in healthy tissue, and they can also grow within the tumor, which suggests that cancer can promote the formation of new lymphatic vessels (lymphangiogenesis). Research has demonstrated that breast cancer patients with a high density of lymphatic vessels had significantly lower rates of disease-free survival and overall survival compared to those with low vessel density. In the tumor's microenvironment, lymphangiogenesis may be triggered by various

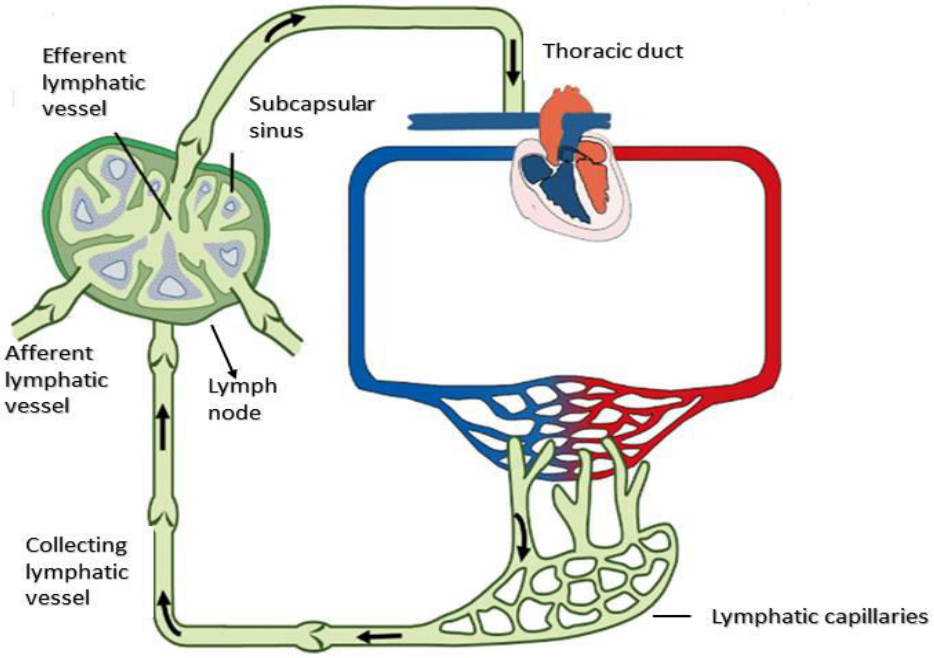


Fig. 1: Lymphatic vessels that carry lymph. Afferent lymphatic vessels carry peripheral tissue fluid into the lymph node, and efferent lymphatic vessels carry that fluid out of the lymph node. Lymph then drains into the lymph node from the afferent lymphatic vessels. After that, lymph fluid moves through the right lymphatic trunk and thoracic duct before entering the venous circulation. The lymph flow direction is shown by arrows.

factors, signaling molecules, and enzymes that are up-regulated (Abe *et al.*, 2016). Figure 1 demonstrates the transportation of lymph through lymph vessels (Shang *et al.*, 2019).

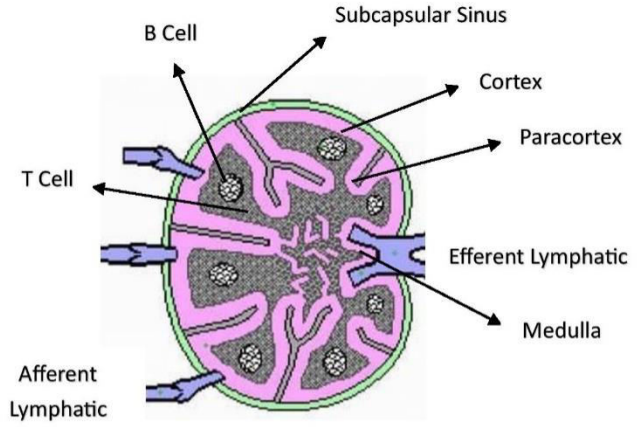
As the lymphatic system, including LNs, is crucial for our immune defense, researchers are becoming more interested in delivering immune molecules like antigens and adjuvants to LNs to induce a strong immune response. In cancer chemotherapy, the primary objective is to deliver drugs specifically and selectively to target organs or cells. Therefore, the development of a new nano-drug delivery system that targets LNs has become an area of intense research interest (Karaman and Detmar, 2014). As a result, LNs have become an appealing therapeutic target for addressing different unmet clinical requirements, such as removing malignant B- and T-cell tumors, treating a reservoir of latent viral-infected cells, managing sentinel lymph node metastases, enhancing vaccines, and promoting immune tolerance (Lorenzo-Redondo *et al.*, 2016). The improved effectiveness of treatment approaches, such as those used in cancer and transplantation, has been demonstrated by targeting specific LNs. Theoretically, drug delivery vehicles that target LNs can enhance the delivery of drugs to LNs, resulting in lower overall doses, reduced off-target effects, and less toxicity (Schudel *et al.*, 2020). Because LNs are locations where immune cells gather and antigen presentation occurs, delivering antigens or immune stimulants to activate systemic anti-tumor immunity is critical. Lymphatic vessels and LNs have become targets for therapy because they are not only common sites for cancer metastasis but also susceptible to pathogens and play a central role in regulating the acquired immune response (Thomas *et al.*, 2016). This emphasizes the importance of developing a dependable method for delivering drugs to the cancer cells present in the lymphatic vessels.

Targeting LNs:

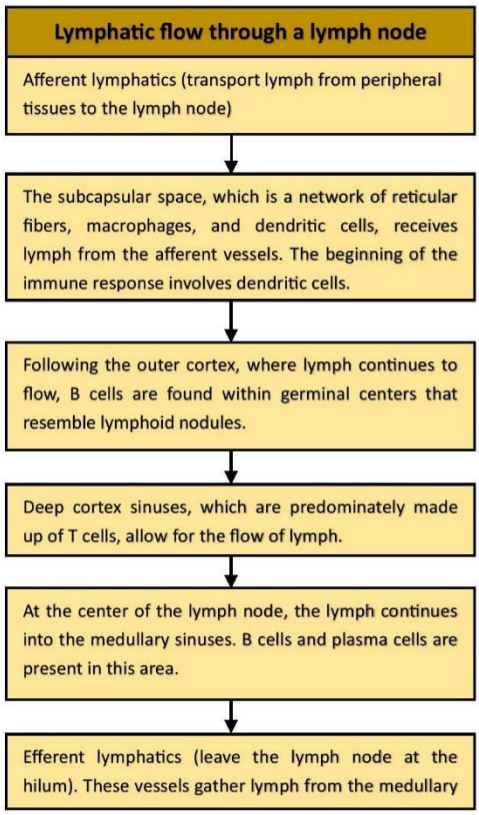
The rationale for targeting LNs is particularly relevant in vaccination, which aims to generate adaptive immunity and immune tolerance. Vaccines are typically administered with immunosuppressive, immunostimulatory, and/or tolerogenic agents that induce signals of tolerance in APCs. APCs include a range of phagocytes with antigen presentation functions, such as professional APCs (dendritic cells, Langerhans cells, macrophages, and B cells) and non-classical APCs with significant stromal functions within LNs. The activation state of APCs and the microenvironment are important for regulating the immune response. DCs are essential for coordinating lymphocyte priming, antigen presentation, processing, and uptake. Macrophages are also important for tissue repair, pathogen clearance, and cytokine and chemokine production. Both DCs and macrophages are targets for designing immunotherapies to enhance immune responses (Schudel *et al.*, 2019). Additionally, macrophages serve as reservoirs for viruses during infection and can have immune-modulatory effects in nearby LNs. As a result, substances are being created to specifically target, modify, or remove macrophages (Ahsan, 2002). Targeting lymphocytes directly is a desirable therapeutic approach because it allows for the regulation of their differentiation, activation, and response to the recognition of an antigen. This approach is particularly important for eliminating lymph node-resident cancers and metastases, such as lymphomas. Lymph node drug delivery is crucial in treating these types of cancers, as well as latent viral reservoirs like HIV that reside within T cells in the LNs and are difficult to treat. Additionally, immunomodulatory agents that act directly on T and B cells can help regulate their function and response to antigen recognition, making them a valuable strategy for vaccination (Ahsan, 2002). Materials engineering can utilize the localization of specific cell subtypes within the LNs to target their endogenous structural features and transport mechanisms. By doing so, it can access both the LNs and the sub-compartments within them that contain these cells. This approach can be highly beneficial in developing targeted delivery systems for therapeutics aimed at treating diseases localized within the LNs. There are also opportunities for materials design in targeting pre-metastatic and metastatic LNs, as these tissues undergo extensive remodeling that alters their structure and may make them more accessible to drugs than healthy LNs (Thomas *et al.*, 2016). For instance, the presence of blood vasculature and abnormal lymphatic (similar to the enhanced permeability and retention effect seen in primary tumors), altered cell phenotypes that are generally more suppressive, and abnormal chemokine and cytokine environments can be exploited as microenvironment-specific features for drug delivery to LNs. This provides the potential for developing lymph node-directed drug delivery systems that can take advantage of these unique features. The structure of a lymph node and the path of lymph flow are shown in Figure 2.

Physiological impediments to LNs drug delivery:

When therapeutic drugs are encapsulated into nanoparticles, the behavior of the nanoparticles in the body is determined by several factors, including their size, surface charge, and composition. These factors can affect the way the nanoparticles interact with biological systems, including the immune system, and ultimately determine the effectiveness of the drug delivery. The size of the nanoparticle is a crucial factor, as it can determine the rate of clearance from the body, as well as the ability to cross biological barriers. Nanoparticles that are too large may be quickly cleared by the immune system, while those that are too small may be rapidly eliminated by the kidneys. Additionally, larger nanoparticles may have difficulty crossing biological barriers, while smaller nanoparticles may be more likely to accumulate in certain organs or tissues (Xiao *et al.*, 2011; Arami *et al.*, 2015; Patten and Shetty, 2018). It is worth noting that deliberate manipulation of the size and charge of nanoparticles can be utilized to overcome specific physiological



A



B

Figure 2. (A) The structure of a lymph node. Most lymph nodes are kidney-shaped sacs that are situated between lymphatic vessels. B cells that produce antibodies are found in the cortex, the lymph node's outermost region. T cells that circulate throughout the body and track immune responses are found in the remaining cortex. The convex side of the lymph node is connected to the afferent lymphatic duct, which carries lymph out through the efferent lymph vessel. (B) path of lymph flow through a lymph node

obstacles and direct the accumulation of nanoparticles in particular tissues, such as the LNs. Nanoparticle carriers that are intended to be delivered to LNs must overcome certain physiological barriers, such as (i) being able to avoid being cleared from the bloodstream by the mononuclear phagocyte system (MPS), (ii) being able to exit fenestrated blood vessels, and (iii) being able to traverse the extracellular matrix (ECM) of the interstitium. Therefore, having a thorough comprehension of these barriers, as well as the size and charge limitations that they impose on nanoparticle design, is crucial for developing effective nanocarrier systems that can successfully target the LNs (Wang *et al.*, 2017; Yuan *et al.*, 2019).

Nanocarriers, after being intravenously administered, encounter their initial obstacle, which is to withstand the clearance process from the bloodstream that is facilitated by macrophages, monocytes of the MPS, and neutrophils that possess the phagocytic capability and have been demonstrated to impact the elimination of nanoparticles *in vivo*. Macrophages are present in major organs, while monocytes and neutrophils are in the bloodstream, and they recognize nanocarriers as foreign due to the adsorption of opsonins on the particle surface, which are recognized by phagocytes through surface receptors (Gustafson *et al.*, 2015). The adsorption of serum proteins onto nanocarrier surfaces leads to phagocytosis and clearance, which has led to research on antifouling polymer coatings like PEG and PCB to prevent this process. The physical properties of nanoparticles, such as size and surface charge, are also important factors in *in vivo* stability and clearance (Fam *et al.*, 2020). The impact of nanoparticle size on phagocyte-mediated clearance has been widely studied, and it is possible to adjust the size to improve nanoparticle circulation. In general, nanoparticles that are smaller than 100 nm tend to experience

less protein adsorption, resulting in longer circulation half-lives (Choi *et al.*, 2011). When designing nanoparticles, it is important to consider surface characteristics such as charge, to prevent their clearance from circulation by phagocytes. Since serum proteins are predominantly negatively charged, nanoparticles with a high positive surface charge (+30 mV) tend to be removed from circulation faster by phagocytes compared to particles that are uncharged or negatively charged (Feng *et al.*, 2018). Metz *et al.* (2004) found that negatively charged iron oxide nanoparticles with a diameter of 21 nm were taken up by phagocytic monocytes at a higher rate than uncharged iron oxide particles of the same size. Surprisingly, they also observed that negatively charged particles with a diameter of 62 nm were phagocytosed at a rate three times higher than uncharged particles with a diameter of 150 nm, indicating that surface charge may have a greater impact on clearance than particle size. These properties determine how well nanoparticles resist removal by phagocytes in the bloodstream, which is important for lymph node targeting strategies as longer-circulating particles have a better chance of accumulating in lymphatic tissues (Metz *et al.*, 2004).

To reach the LNs, nanoparticles must not only avoid phagocytic clearance but also exit the bloodstream by extravasating through the endothelium. The endothelium is composed of flat, inactive cells connected by tight junction proteins and a basement membrane containing various proteins. The blood vessels act as a barrier that limits the movement of fluids, nanoparticles, and other materials between the interstitial and intravascular compartments. Before extravasation can occur, nanoparticles must move toward the blood vessel walls, and studies suggest that ellipsoid or rod-shaped particles with higher aspect ratios can marginate better than spherical particles. Once they reach the vessel wall, nanoparticles must exit through fenestrations or transcellular transport. This process is crucial for effective lymph node targeting (Toy *et al.*, 2011; Rodrigues and Granger, 2015). Fenestrations present in the endothelium of blood vessels can range from 60 nm to 800 nm in the case of tumor vasculature, and they are thought to be a primary route for nanoparticles smaller than 200 nm to extravasate from the bloodstream into the interstitium. As a result, reducing the size of nanoparticles has been shown to increase their extravasation out of the blood vessel endothelium. For instance, human umbilical vein endothelial cells (HUVECs) were used as an *in vitro* model of the blood vasculature and there was more than a 10-fold increase in the extravasation of 40 nm polystyrene nanoparticles from the blood endothelium compared to 70 nm and 130 nm particles (Thomas and Weber, 2019; Vu *et al.*, 2019). After leaving the bloodstream, nanoparticles may face a new obstacle in the interstitium, which is the ECM, before reaching the LNs. The ECM is a complex network of proteins produced mainly by fibroblasts, such as collagen, glycosaminoglycans, laminins, and fibronectin. Collagen fibrils crosslink to create a mesh-like structure with pore sizes that can vary from 20 nm to 130 nm. Because of the variable pore sizes of the ECM, smaller nanoparticles are believed to have greater mobility through the ECM (Yohan *et al.*, 2016; Engin *et al.*, 2017).

Lipid-Based Lymphatic Targeting:

The lymphatic system plays a crucial role in transferring fats from the digestive system into the bloodstream. Normally, lipids enter specialized cells called enterocytes in the gastrointestinal tract either through a process called receptor-mediated endocytosis or micropinocytosis (Chaudhary *et al.*, 2014). Chylomicron-packaged fats are released from the enterocyte cell's basolateral side into the lamina propria within the gastrointestinal epithelium. The thoracic duct, which empties into the subclavian vein, is where chylomicrons first enter the lymphatic vessels before entering the bloodstream. There are a number of distinctive opportunities for developing drug delivery systems provided by this chylomicron processing

pathway, as it provides a means of accessing LNs to enhance immunotherapies and enables systemic delivery of orally administered drugs while bypassing hepatic first pass metabolism. To optimize the utilization of the chylomicron processing pathway for delivering drugs through the lymphatic system, it is possible to design therapeutics as prodrugs containing a lipid component that can be cleaved, thereby triggering this natural transport pathway. Numerous studies have extensively documented how lipid formulations that mimic triglycerides, as well as other fatty acids, have the potential to enhance the delivery of various drugs to lymphatic vessels and LNs connected to the gut, particularly when administered orally (Kochappan *et al.*, 2021; Lee *et al.*, 2021). The chylomicron pathway has been used by some research teams to deliver particulate cargo to the intestinal lymphatics. Mao and colleagues, for example, developed mesoporous silica nanoparticles measuring between 100 and 120 nm in size, which were coated with diglycerides in order to trigger the chylomicron processing pathway. They provided evidence that lipases present in the gastrointestinal lumen hydrolyzed the fats coating the surface of the nanoparticles, leading to their uptake and subsequent processing into chylomicrons. This facilitated their transportation into the lymphatic vessels (Mao *et al.*, 2019). *et al.* (2021b) conducted a study to investigate the impact of adding a prodrug to their nanoparticle formulation on the delivery to lymphatic vessels. They used Orlistat as a lipophilic drug and formulated emulsions with different types of fatty acids. The highest concentration of Orlistat in lymphatic vessels was achieved when coated with long-chain fatty acids, surpassing the concentrations achieved with short-chain fatty acids and triglycerides. After oral administration, the drug's lymph concentration peaked 2-3 h later. Furthermore, fatty acid concentrations on the emulsion's surface increased the transport of nanoparticles through intestinal epithelial barriers and facilitated their entry into lymphatic vessels (Lee *et al.*, 2021). Kochappan *et al.* (2021) recently developed a formulation where mycophenolic acid (MPA) was attached to triglycerides. Their aim was to utilize the chylomicron processing pathway to enhance the delivery of the immunomodulatory drug to LNs and lymphatic vessels. When compared to MPA given alone or in combination with other agents, the MPA-fatty acid conjugate showed improved drug concentration in the lymph after intraduodenal administration. Notably, the MPA-fatty acid conjugate exhibited a 20-fold higher concentration of MPA within the LNs compared to MPA delivered independently (Kochappan *et al.*, 2021).

Therapeutic Targets for LECs:

The lymphatic endothelium, by communicating with immune cells, also plays a significant role in immunity in addition to its functions in transportation and fluid regulation. LECs are distributed throughout the body and produce important chemokines and adhesion molecules that contribute to the immune-modulating functions of lymphatic vessels.

LECs-Mediated Immunity:

Lymphatic vessels serve as a pathway for immune cells, allowing them to enter peripheral lymphatic vessels within tissues and migrate to nearby LNs. Dendritic cells, neutrophils, monocytes, B cells, and T cells, among others, rely on lymphatic vessels for their migration. This migration is often facilitated by the CCL21-CCR7 axis, a chemokine receptor interaction that helps guide immune cell movement (Kriehuber *et al.*, 2001). CCR7 plays a vital role in immune cell migration to LNs and is also involved in guiding DCs to various tissues such as the lamina propria, lungs, and skin. It has been observed that CCR7 regulates the association of DCs with collecting lymphatic vessels, leading to increased vessel permeability (Hampton and Chtanova, 2019). DCs show a preference for entering lymphatic vessels at locations with a high concentration gradient of CCL21, indicating that CCL21 plays a direct role in regulating entry into

lymphatics. It has been shown by intravital microscopy that CCL21 gradients promote dendritic cell migration inside lymphatic vessels. Moreover, CCL21 secreted by LECs also plays a role in guiding naïve T cells from distant peripheral tissues to LNs (Farnsworth *et al.*, 2019). In addition, LEC (lymphatic endothelial cell) interaction with DCs goes beyond their role in regulating dendritic cell migration via the CCL21-CCR7 axis. It has been discovered that LECs can directly influence dendritic cell maturation. Studies have shown that LECs can inhibit dendritic cell maturation through interactions involving ICAM-1/Mac-1, as well as through the synthesis of anti-inflammatory prostacyclin and the secretion of TGF-β. These interactions and secretions by LECs contribute to the modulation of dendritic cell function and maturation (Garnier *et al.*, 2019). LEC modulation of immune responses includes a fascinating mechanism wherein they transfer antigens to professional antigen-presenting cells, as well as to CD4+ and CD8+ cells. This process allows LECs to actively participate in antigen presentation and the activation of specific immune cell populations. Tamburini *et al.* (2014) conducted a study showing that LEC proliferation combined with antigen capture resulted in prolonged antigen expression within LECs. This led to increased production of IL-2 and IFNγ, ultimately improving infection protection. According to the study, CD8+ and CD4+ T cell responses were dysfunctionally activated when delivering MHCI and MHCII-restricted antigens, but the specific role of LEC antigen presentation in this context remains unclear (Tamburini *et al.*, 2014).

Immunomodulation by Specifically Targeting Lymphatic Vessels:

Lymphatic vessels have gained attention as a promising therapeutic target due to their significant role in immunomodulation. One approach to potentially target lymphatic vessels is by focusing on specific receptors expressed on these vessels, such as VEGFR-3 or Lyve-1. VEGFR-3, belonging to the vascular endothelial growth factor receptor family, is particularly known for its involvement in promoting lymphangiogenesis (Witmer *et al.*, 2001). Lyve-1, also known as lymphatic vessel endothelial hyaluronan receptor 1, is a widely used integral membrane protein that serves as a marker for identifying LECs. However, it is worth noting that Lyve-1 can also be present in other tissues such including specific subsets of macrophages, embryonic vessels, and liver blood sinusoids (Brezovakova and Jadhav, 2020). Guo *et al.* (2013) conducted an *in vitro* study where they specifically targeted LECs by utilizing Lyve-1 as a targeting mechanism. They employed Lyve-1-binding PEG (polyethylene glycol) to ultrasmall superparamagnetic iron oxide nanoparticles, aiming to develop a potential contrast agent for magnetic resonance imaging (MRI) (Guo *et al.*, 2013). Dashkevich *et al.* (2016) utilized the targeting of VEGFR-3 to effectively prevent cardiac allograft rejection. Their study revealed that inhibiting VEGFR-3 resulted in a decrease in the production of CCL21, a chemokine that plays a crucial role in immune cell migration (Dashkevich *et al.*, 2016). They discovered that the decrease in CCL21 production did not have an impact on lymphangiogenesis within the graft, but it did lead to lower levels of CD8+ T cells present in the graft. In a different experiment, they showed that administration of a neutralising monoclonal antibody targeting VEGFR-3 resulted in reduced arteriosclerosis, decreased expression of VEGFR-3 and CCL21 in activated lymphatic vessels, and a decrease in CD4+ T cells infiltrating chronically rejecting mouse cardiac allografts. By hindering the interaction between VEGF-C produced by tumours and the lymphatic vessel VEGFR-3, this approach holds potential as a means of suppressing tumor growth and metastasis by inhibiting tumor lymphangiogenesis (Su *et al.*, 2007). Research in the field of cancer has shown that administering VEGF-C, the ligand that activates VEGFR-3, can enhance the efficacy of immunotherapy treatments in cases of melanoma (Yeh *et al.*, 2017). Fankhauser *et al.* (2017) conducted a study where they provided evidence that VEGF-C, despite its role in promoting angiogenesis and metastasis, also enhances the

effectiveness of immunotherapy by attracting naïve T cells. The research group also showed that patients who positively responded to immunotherapy exhibited the expression of lymphatic-related markers within their tumors. This correlation suggests that lymphatic vessels play a role in augmenting the effectiveness of tumor immunotherapies (Fankhauser *et al.*, 2017).

Targeting LNs using in vivo application of nanoparticles:

The limitations of the mononuclear phagocyte system, blood vessel endothelium, and ECM of the interstitium dictate that smaller, uncharged nanoparticles may have better efficacy in targeting the LNs after being administered intravenously. This has resulted in the use of nanoparticle delivery to the LNs in various clinical and preclinical settings, including for the treatment and imaging of lymph node cancer metastases, as well as for vaccine delivery (Chida *et al.*, 2018). Typically, intravenous administration of nanoparticle formulations results in their accumulation in the liver and spleen, with lymph node concentrations of less than 0.1% of the injected dose. However, modifying the physical properties of nanoparticles, such as their size and charge, has been demonstrated to increase their accumulation in the LNs (Elgrabli *et al.*, 2015). Dextran-coated ultrasmall superparamagnetic iron oxide (USPIO) nanoparticles with hydrodynamic diameters of approximately 40 nm have been clinically tested for identifying lymph node metastases in prostate cancer using MRI. Preclinical studies have reported up to 12% ID in the LNs after intravenous administration in rats. These nanoparticles function as contrast agents and are retained by macrophages in the LNs. In a clinical study with 80 patients and 334 LNs, USPIO-aided MRI improved the sensitivity of lymph node metastases detection from 35.4% to 90.5% compared to MRI alone (Harisinghani *et al.*, 2003).

Nanoparticles that are specifically designed to target the LNs have been studied not only for imaging cancer, but also for treating it. Cabral *et al.* (2015) conducted research on uncharged polymeric micelles that were administered intravenously to mice bearing B16F10 tumors, and studied their ability to accumulate in metastatic LNs and deliver a platinum-based drug (Cabral *et al.*, 2015). In a study conducted by Cabral *et al.*(2015) mice were treated with free oxaliplatin or oxaliplatin loaded into 30 nm polymeric micelles. Mice treated with the drug-loaded micelles had significantly smaller tumors and lymph node metastases compared to untreated mice or mice treated with free oxaliplatin. The accumulation of the micelles in lymph node metastases was found to be size-dependent, with 30 nm micelles having approximately 4 times better accumulation than 70 nm micelles. In another study by the same group, 55 nm micelles were investigated for their ability to deliver the chemotherapy drug epirubicin to metastatic LNs in a murine breast cancer model expressing luciferase (Chida *et al.*, 2018). To minimize the risk of off-target toxicity due to drug release in healthy LNs, the research team used pH-sensitive hydrazine linkers to load epirubicin into the micelles. This allowed for increased drug release in the acidic environment of the primary tumor and lymph node metastases. The micelle-treated mice had 10-fold higher epirubicin concentration in metastatic LNs 8 h after intravenous injection compared to mice treated with free epirubicin. This resulted in a significant reduction in the size of lymph node metastases. Furthermore, the drug concentration in metastatic LNs was almost 3-fold higher than the drug concentration in healthy nodes 48 h after injection. Solid cancers tend to spread through the lymphatics, and thus, nanoparticles targeting LNs have been investigated to inhibit the formation of lymph node metastases. Liu *et al.* (2019) used 5 nm dendrimer-loaded cisplatin prodrug to assemble nanoparticle clusters of 100 nm size and tested their efficacy in inhibiting lymph node metastases. These nanoparticles were injected intravenously and found to enter the lymphatics, as confirmed by colocalization with FITC-labeled lymphatic vessels. Furthermore, the use of these

nanoparticles inhibited lymph node metastases in a metastatic 4T1 breast cancer model and improved the median survival of mice from 45 to 56 days, which validates the effectiveness of lymph node-targeted nanoparticles for cancer therapy.

Nanoparticles have been investigated for their potential to deliver vaccines to the LNs for cancer immunization. Baharom *et al.* (2021) studied the use of 20-50 nm micelles loaded with MC38 tumor peptide neoantigens for anti-tumor vaccination of mice (Baharom *et al.*, 2021). The study found that mice challenged with MC38 colon cancer cells after vaccination had significantly reduced tumor growth compared to unvaccinated mice. Interestingly, the study also found that intravenous vaccination was effective in controlling tumor growth in mice with established MC38 tumors, while subcutaneous vaccination was ineffective. These findings indicate the success of small, uncharged nanoparticles for lymph node targeting, which aligns with the ideal nanoparticle physical properties. The increasing number of studies on lymph node drug delivery for cancer therapy demonstrates the growing understanding of the role of the lymphatic system in cancer metastasis and recurrence.

Hitchhiking techniques for LNs drug delivery:

Albumin-facilitated transportation of drugs to the LNs:

A different approach to delivering drugs to the LNs is to create a delivery system that can attach to molecules or immune cells that naturally travel to the LNs. Albumin is a protein found in blood, interstitial fluid, and lymph, and it is the most abundant protein in these fluids. The liver produces albumin, which has a globular shape and dimensions of 4x15 nanometers, and an average lifespan of 19 days (Merlot *et al.*, 2014). Albumin helps maintain the osmotic pressure of blood and carries other biomolecules such as hormones, fatty acids, and steroids. Approximately 5% of the albumin in circulation exits the blood vessels into the interstitial space every hour, and nearly all this albumin is taken up by the lymphatic vessels before re-entering circulation (Abdallah *et al.*, 2020). Albumin's stability and frequent trafficking to the lymphatics make it an attractive candidate for subcutaneous delivery systems. These systems utilize albumin-hitchhiking for the delivery of imaging molecules, such as Evans Blue, and vaccines to draining LNs. Additionally, albumin has been studied as a nanocarrier for anticancer therapies, as it can passively accumulate in primary tumors through leaky tumor vasculature and actively transport into tumor cells via the Cav-1 protein (Yao *et al.*, 2018).

In preclinical and clinical studies, albumin hitchhiking has been used to create intravenous delivery systems that specifically target the LNs. For instance, researchers like Yu *et al.* (2017) have successfully created combination nanoparticles by loading nanospheres composed of crosslinked albumin with gemcitabine (a chemotherapy drug) and pheophorbide-a (a photodynamic therapy agent) to evaluate its efficacy in treating lymph node metastases in a murine BxPC-3 pancreatic cancer model. After 24 h of intravenous administration, the combination nanoparticle showed higher accumulation in metastatic LNs than free pheophorbide-a. Furthermore, *in vivo* efficacy studies indicated that the mice treated with the nanoparticle had reduced volume and weight of metastatic LNs. These results suggest that albumin hitchhiking may be a promising strategy for targeted drug delivery to LNs and treating metastatic cancer, but further research is necessary to fully explore its clinical potential.

Abraxane, which is a type of medication for fighting cancer, contains paclitaxel that is bound to albumin in a nano-sized formulation. It has been granted approval by the FDA to be used in the treatment of metastatic breast cancer, non-small cell lung cancer, and pancreatic adenocarcinoma (Kundranda and Niu, 2015). Due to its ability to target the lymphatic system, Abraxane has been recently investigated for its potential use in cancers that are located in the LNs, such as non-Hodgkin lymphoma. Two cases have been

reported where patients with diffuse large B-cell lymphoma showed only partial responses to multiple rounds of chemotherapy, but were later treated with combination therapies that included nab-paclitaxel. The first patient received weekly doses of nab-paclitaxel for six months after being treated with the chemotherapy drug azacitidine, and PET-CT scans revealed complete remission of the disease after three months of treatment (Bowen *et al.*, 2017). Follow-up scans conducted five years after the treatment showed no signs of the disease returning. The second patient was given a combination of nab-paclitaxel and liposomal doxorubicin as a fourth-line chemotherapy, and achieved complete remission of the disease after four treatment cycles. PET-CT scans conducted seven years after the treatment showed no signs of disease recurrence (Wang and Xia, 2013). Out of the 13 patients involved in the study, 5 experienced a complete response to the treatment, 6 had a partial response, while 2 patients showed stable or progressive disease. Although previous clinical studies had reported successful therapeutic outcomes in patients who had not responded to other chemotherapy treatments, these studies were limited by their small sample sizes. Therefore, further investigation is required to determine the effectiveness of using nab-paclitaxel alone as a treatment for diffuse large B cell lymphoma.

Drug delivery to the LNs by immune cells:

One alternative method for delivering medications to the LNs involves attaching nanoparticles to immune cells that frequently travel to the LNs, such as T cells, monocytes, and dendritic cells. These immune cells can enter the LNs through both the lymphatic vessels and blood vessels. Naive T cells continuously move between the blood circulation and lymphatic system in search of antigens. When activated, T cells produce chemokine receptors such as CCR5, CCR7, and CCR8, which enable them to migrate to the LNs through the lymphatic and blood vessels within the LNs (Martens *et al.*, 2020). Huang *et al.* (2015) utilized T cells' ability to home in on LNs by linking cultured T cells that produce luciferase to tiny lipid nanocapsules (NCs) containing SN-38, an anticancer drug. After injecting these T cells with NCs into a mouse model with lymphoma, they observed that the T cells conjugated with the NCs accumulated in the LNs within 20 h, reaching their peak levels at 60 h post-injection, and remained there until the end of the study (80 h). This demonstrated that the conjugation of T cells to NCs did not affect the trafficking of T cells. Tumor-bearing LNs were evaluated to determine drug concentrations, and it was found that T cell-mediated NC delivery resulted in an increase in drug concentration that was over 60 times greater than the concentration achieved by injecting unbound NCs. This increased drug concentration led to a significant reduction in tumor burden, approximately 10 times more than the unbound NC treatment.

Furthermore, achieving T cell hitchhiking within the body is possible. As an illustration, Schmid *et al.* (2017) investigated the capability of T cell-targeted nanoparticles made of PEG-PLGA, which were administered intravenously, to accumulate within the LNs that drain the tumor in a mouse model of colorectal cancer. These nanoparticles were used to deliver SD-208, an immunomodulatory drug that works by obstructing the TGF-BR1 kinase in cancer cells, which is known to trigger immunosuppression (Uhl *et al.*, 2004; Schmid *et al.*, 2017). Similar to T cells, DCs are immune cells that travel to the LNs and play a crucial role in mediating adaptive immunity. Being the primary APCs of the immune system, DCs are distributed throughout the body and are usually found in higher concentrations in tissues that are exposed to the external environment, such as the skin, intestinal lining, and nasal passages. Although DCs are known to migrate to the LNs to some extent during steady-state conditions, most of the DCs migration occurs after antigen exposure, during which DCs start to express surface receptors like CCR7. These receptors are used to track chemokines such as CCL19 and CCL21 to the lymphatics, where DCs

start to initiate adaptive immune responses through antigen presentation to naïve T cells and B cells (Alvarez *et al.*, 2008; Jia *et al.*, 2018).

Apart from T cells and DCs, monocytes are a type of immune cells that swiftly gather in the LNs via blood vessels during and shortly after inflammation. They serve various purposes such as transporting and presenting antigens and transforming into DCs. However, it has been noted that they can also be found in stable conditions (Jakubzick *et al.*, 2013; Teh *et al.*, 2019). The arrival of monocytes during periods of inflammation has been documented as being caused by the build-up of chemokines, specifically monocyte chemoattractant protein-1 (MCP-1), in the LNs. These chemokines enter the LNs through the afferent lymphatic vessels (Palframan *et al.*, 2001). The heightened movement of monocytes to the LNs in inflammatory conditions like cancer offers a valuable strategy for delivering drugs specifically to the LNs through monocyte targeting. An investigation in this field involved the creation of micelles that were customized with peptides obtained from the CCR2-binding motif present in the MCP-1 chemokine. These micelles were administered intravenously to mice with B16F10 tumors. It was observed that the micelles containing the CCR2-binding motif accumulated in the LNs at a rate twice as fast as non-targeted micelles, three hours after injection. However, additional research is needed to fully understand the underlying mechanisms responsible for this phenomenon (Trac *et al.*, 2021). The creation of nanoparticle systems that have a strong affinity for immune cells traveling through the lymphatics provides a new and innovative approach for delivering drugs to the LNs.

Conclusion

The physiological characteristics and cellular mechanisms involved in the functioning of LNs provide valuable insights for the development of targeted materials that can effectively interact with specific cell types within the LNs. A crucial aspect of this process is the utilization of afferent lymphatics, which serve as entry points for nanomaterials to transport cargo to cells present in the LNs. These cells include dendritic cells, macrophages, and B cells, which play essential roles in immune response and antigen presentation within the LNs. By understanding and harnessing these transport mechanisms, it becomes possible to design materials that can precisely target and interact with specific cell populations in the LNs. Nanoparticles offer numerous benefits for drug delivery to LNs. Their larger size compared to typical biomolecules allows for easy migration into lymphatic vessels. Furthermore, nanoparticles can be modified on their surfaces to specifically target LNs. However, when introduced in this manner, nanoparticles may encounter interactions with phagocytic cells in the sinus, and their entry into the T cell zone may be challenging due to limitations imposed by their size.

In this review, we began by introducing the fundamental structure and function of LNs. However, our main emphasis was on the emerging field of LN-targeted nano-drug-delivery systems. The progress and translation of targeted delivery systems for LNs depend on the development of basic research in related areas, including a comprehensive understanding of the diverse cell types present in LNs and the intricate cell-to-cell communication that occurs between LNs and remote tissues. It is important to note that the lymph node holds significant potential as a compelling target for immune therapy and vaccine development in future clinical trials. As such, further research in this area is warranted to explore its full potential.

LNs play a crucial role in immune response initiation as they facilitate the interaction between peripheral immune signals and circulating lymphocytes. Therefore, LNs' physiological and structural features, including their transport mechanisms, should guide the development of drug delivery systems. A comprehensive understanding of the various cell types, cell-cell interactions, and drug mechanisms of action within the lymph node can provide opportunities

to target specific regions and cells in the lymph node.

References

Abdallah M, Müllertz OO, Styles IK, Mörsdorf A, Quinn JF, Whittaker MR and Trevaskis NL. (2020) Lymphatic targeting by albumin-hitchhiking: Applications and optimisation. J Control Release 327: 117-128.

Abe N, Ohtake T, Saito K, Kumamoto K, Sugino T and Takenoshita, S. (2016) Clinicopathological significance of lymphangiogenesis detected by immunohistochemistry using D2-40 monoclonal antibody in breast cancer. Fukushima J Med Sci. 62(1): 57-63.

Ahsan F. (2002) Targeting to macrophages: role of physicochemical properties of particulate carriers—liposomes and microspheres—on the phagocytosis by macrophages. J Control Release 79(1–3): 29-40.

Alvarez D, Vollmann EH and von Andrian UH. (2008) Mechanisms and consequences of dendritic cell migration. Immunity 29(3): 325-342.

Arami H, Khandhar A, Liggitt D and Krishnan KM. (2015) *In vivo* delivery, pharmacokinetics, biodistribution and toxicity of iron oxide nanoparticles. Chem Soc Rev. 44(23): 8576-8607.

Baharom F, Ramirez-Valdez RA, Tobin KKS, Yamane H, Dutertre CA, Khalilnezhad A, Reynoso GV, Coble VL, Lynn GM, Mule MP, Martins AJ, Finnigan JP, Zhang XM, Hamerman JA, Bhardwaj N, Tsang JS, Hickman HD, Ginhoux F, Ishizuka AS and Seder RA. (2021) Intravenous nanoparticle vaccination generates stem-like TCF1^{+} neoantigen-specific CD8^{+} T cells. Nat Immunol 22: 41-52.

Bowen RC, Hahn AW, Butler TW and Khong HT. (2017) Complete response to azacitidine priming and nab-paclitaxel in non-Hodgkin lymphoma resistant to biochemotherapy. Mol Clin Oncol. 6(1): 122-124.

Brezovakova V and Jadhav S. (2020) Identification of Lyve-1 positive macrophages as resident cells in meninges of rats. J Comp Neurol. 528(12): 2021–2032.

Cabral H, Makino J, Matsumoto Y, Mi P, Wu H, Nomoto T, Toh K, Yamada N, Higuchi Y, Konishi S, Kano MR, Nishihara H, Miura Y, Nishiyama N and Kataoka K. (2015) Systemic targeting of lymph node metastasis through the blood vascular system by using size-controlled nanocarriers. ACS Nano. 9: 4957-4967.

Chaudhary S, Garg T, Murthy RSR, Rath G and Goyal AK. (2014) Recent approaches of lipid-based delivery system for lymphatic targeting via oral route. J Drug Targeting 22(10): 871-882.

Chida T, Miura Y, Cabral H, Nomoto T, Kataoka K and Nishiyama N. (2018) Epirubicin-loaded polymeric micelles effectively treat axillary lymph nodes metastasis of breast cancer through selective accumulation and pH-triggered drug release. J Control Release 292: 130-140.

Choi CHJ, Zuckerman JE, Webster P and Davis ME. (2011) Targeting kidney mesangium by nanoparticles of defined size. Proc Nat Acad Sci. 108(16): 6656-6661.

Cote B, Rao D, Alany RG, Kwon GS and Alani AWG. (2019) Lymphatic changes in cancer and drug delivery to the lymphatics in solid tumors. Adv Drug Deliv Rev. 144: 16-34.

Dashkevich A, Raissadati A, Syrjälä SO, Zarkada G, Keränen MAI, Tuuminen R, Krebs R, Anisimov A, Jeltsch M, Leppänen VM, Alitalo K, Nykänen AI and Lemström KB. (2016) Ischemia–reperfusion injury enhances lymphatic endothelial VEGFR3 and rejection in cardiac allografts. American J Transplantation 16(4): 1160-1172.

Dieterich LC, Seidel CD and Detmar M. (2014) Lymphatic vessels: new targets for the treatment of inflammatory diseases. Angiogenesis 17(2): 359–371.

Elgrabli D, Beaudouin R, Jbilou N, Floriani M, Pery A, Rogerieux F and Lacroix G. (2015) Biodistribution and clearance of TiO_2 nanoparticles in rats after intravenous injection. PLoS One 10(4): e0124490.

Engin AB, Nikitovic D, Neagu M, Henrich-Noack P, Docea AO, Shtilman MI, Golokhvast K and Tsatsakis AM. (2017) Mechanistic understanding of nanoparticles' interactions with extracellular matrix: The cell and immune system. In: Particle and Fibre Toxicology. https://doi.org/10.1186/s12989-017-0199-z

Fam SY, Chee CF, Yong CY, Ho KL, Mariatulqabtiah AR and Tan WS. (2020) Stealth coating of nanoparticles in drug-delivery systems. Nanomaterials 10(4): 787.

Fankhauser M, Broggi MAS, Potin L, Bordry N, Jeanbart L, Lund AW, Da Costa E, Hauert S, Rincon-Restrepo M, Tremblay C, Cabello E, Homicsko K, Michielin O, Hanahan D, Speiser DE and Swartz MA. (2017) Tumor lymphangiogenesis promotes T cell infiltration and potentiates immunotherapy in melanoma. Sci Transl Med. 9(407): eaal4712.

Farnsworth RH, Karnezis T, Maciburko SJ, Mueller SN and Stacker SA. (2019) The interplay between lymphatic vessels and chemokines. Front Immunol. 10: 518.

Feng Q, Liu Y, Huang J, Chen K, Huang J and Xiao K. (2018) Uptake, distribution, clearance, and toxicity of iron oxide nanoparticles with different sizes and coatings. Sci Rep. 8: 2082.

Garnier L, Gkountidi AO and Hugues S. (2019) Tumor-

associated lymphatic vessel features and immunomodulatory functions. Front Immunol. 10: 720.

Gerner MY, Torabi-Parizi P and Germain RN. (2015) Strategically localized dendritic cells promote rapid T cell responses to lymph-borne particulate antigens. Immunity 42(1): 172-185.

Guo KX, Ke R, WenGe S and Yi L. (2013) Mouse lymphatic endothelial cell targeted probes: anti-LYVE-1 antibody-based magnetic nanoparticles. Int J Nanomedicine 8: 2273-2284.

Gustafson HH, Holt-Casper D, Grainger DW and Ghandehari H. (2015) Nanoparticle uptake: The phagocyte problem. Nano Today 10(4): 487-510.

Hampton HR and Chtanova T. (2019) Lymphatic migration of immune cells. Front Immunol. 10: 1168.

Harisinghani MG, Barentsz J, Hahn PF, Deserno WM, Tabatabaei S, van de Kaa CH, de la Rosette J and Weissleder R. (2003) Noninvasive detection of clinically occult lymph-node metastases in prostate cancer. New Eng J Med. 348(25): 2491-2499.

Huang B, Abraham WD, Zheng Y, Bustamante López SC, Luo SS and Irvine DJ. (2015) Active targeting of chemotherapy to disseminated tumors using nanoparticle-carrying T cells. Sci Transl Med. 7(291): 291ra94.

Jakubzick C, Gautier EL, Gibbings SL, Sojka DK, Schlitzer A, Johnson TE, Ivanov S, Duan Q, Bala S, Condon T, van Rooijen N, Grainger JR, Belkaid Y, Ma'ayan A, Riches DWH, Yokoyama WM, Ginhoux F, Henson PM and Randolph GJ. (2013) Minimal differentiation of classical monocytes as they survey steady-state tissues and transport antigen to lymph nodes. Immunity 39(3): 599-610.

Jalkanen S and Salmi M. (2020a) Lymphatic endothelial cells of the lymph node. Nature Rev Immunol. 20(9): 5660578.

Jarjour M, Jorquera A, Mondor I, Wienert S, Narang P, Coles MC, Klauschen F and Bajénoff M. (2014) Fate mapping reveals origin and dynamics of lymph node follicular dendritic cells. J Exp Med. 211(6): 1109-1122.

Jia J, Zhang Y, Xin Y, Jiang C, Yan B and Zhai S. (2018) Interactions between nanoparticles and dendritic cells: From the perspective of cancer immunotherapy. Front Oncol. 8: 404.

Karaman S and Detmar M. (2014) Mechanisms of lymphatic metastasis. J Clin Invest. 124(3): 922-928.

Kato S, Shirai Y, Sakamoto M, Mori S and Kodama T. (2019) Use of a lymphatic drug delivery system and sonoporation to target malignant metastatic breast cancer cells proliferating in the marginal sinuses. Sci Rep. 9(1): 13242.

Kato, S., Takeda, K., Sukhbaatar, A., Sakamoto, M., Mori, S., Shiga, K., and Kodama, T. (2020). Intranodal pressure of a metastatic lymph node reflects the response to lymphatic drug delivery system. *Cancer Science*, *111*(11), 4232–4241.

Kochappan R, Cao E, Han S, Hu L, Quach T, Senyschyn D, Ferreira VI, Lee G, Leong N, Sharma G, Lim SF, Nowell CJ, Chen Z, von Andrian UH, Bonner D, Mintern JD, Simpson JS, Trevaskis NL and Porter CJH. (2021) Targeted delivery of mycophenolic acid to the mesenteric lymph node using a triglyceride mimetic prodrug approach enhances gut-specific immunomodulation in mice. J Controlled Release 332: 636-651.

Kriehuber E, Breiteneder-Geleff S, Groeger M, Soleiman A, Schoppmann SF, Stingl G, Kerjaschki D and Maurer D. (2001) Isolation and characterization of dermal lymphatic and blood endothelial cells reveal stable and functionally specialized cell lineages. J Exp Med. 194(6): 797-808.

Kundranda MN and Niu J. (2015) Albumin-bound paclitaxel in solid tumors: Clinical development and future directions. Drug Des Devel Ther. 9: 3767-3777.

Lee G, Han S, Lu Z, Hong J, Phillips ARJ, Windsor JA, Porter CJH and Trevaskis NL. (2021). Intestinal delivery in a long-chain fatty acid formulation enables lymphatic transport and systemic exposure of orlistat. Intl J Pharmaceut. 596: 120247.

Liao S and von der Weid PY. (2015) Lymphatic system: An active pathway for immune protection. Semin Cell Dev Biol. 38: 83-89.

Liu J, Li HJ, Luo YL, Xu CF, Du XJ, Du JZ and Wang J. (2019) Enhanced primary tumor penetration facilitates nanoparticle draining into lymph nodes after systemic injection for tumor metastasis inhibition. ACS Nano 13(8): 8648-8658.

Lorenzo-Redondo R, Fryer HR, Bedford T, Kim EY, Archer J, Kosakovsky Pond SL, Chung YS, Penugonda S, Chipman JG, Fletcher CV, Schacker TW, Malim MH, Rambaut A, Haase AT, McLean AR and Wolinsky SM. (2016) Persistent HIV-1 replication maintains the tissue reservoir during therapy. Nature 530(7588): 51-56.

Lund AW, Duraes FV, Hirosue S, Raghavan VR, Nembrini C, Thomas SN, Issa A, Hugues S and Swartz MA. (2012) VEGF-C promotes immune tolerance in B16 melanomas and cross-presentation of tumor antigen by lymph node lymphatics. Cell Rep. 1(3): 191-199.

Mao Y, Feng S, Li S, Zhao Q, Di D, Liu Y and Wang S. (2019) Chylomicron-pretended nano-bio self-

assembling vehicle to promote lymphatic transport and GALTs target of oral drugs. Biomaterials 188: 173-186.

Margaris KN and Black RA. (2012) Modelling the lymphatic system: Challenges and opportunities. J R Soc Interface. 9(69): 601-612.

Martens R, Permanyer M, Werth K, Yu K, Braun A, Halle O, Halle S, Patzer GE, Bošnjak B, Kiefer F, Janssen A, Friedrichsen M, Poetzsch J, Kohli K, Lueder Y, Gutierrez Jauregui R, Eckert N, Worbs T, Galla M and Förster R. (2020) Efficient homing of T cells via afferent lymphatics requires mechanical arrest and integrin-supported chemokine guidance. Nat Commun. 11(1): 1114.

Mebius RE, Streeter PR, Brevé J, Duijvestijn AM and Kraal G. (1991) The influence of afferent lymphatic vessel interruption on vascular addressin expression. J Cell Biol. 115(1): 85-95.

Merlot AM, Kalinowski DS and Richardson DR. (2014) Unraveling the mysteries of serum albumin-more than just a serum protein. Front Physiol. 5: 299.

Metz S, Bonaterra G, Rudelius M, Settles M, Rummeny EJ and Daldrup-Link HE. (2004) Capacity of human monocytes to phagocytose approved iron oxide MR contrast agents in vitro. Eur Radiol. 14(10): 1851-1858.

Moran I, Grootveld AK, Nguyen A and Phan TG. (2019) Subcapsular sinus macrophages: The seat of innate and adaptive memory in murine lymph nodes. Trends Immunol. 40(1): 35-48.

Palframan RT, Jung S, Cheng G, Weninger W, Luo Y, Dorf M, Littman DR, Rollins BJ, Zweerink H, Rot A and von Andrian UH. (2001) Inflammatory chemokine transport and presentation in HEV. J Exp Med. 194(9): 1361-1374.

Patten DA and Shetty S. More than just a removal service: scavenger receptors in leukocyte trafficking. Front Immunol. 9: 2904.

Proulx ST, Luciani P, Dieterich LC, Karaman S, Leroux JC and Detmar M. (2013) Expansion of the lymphatic vasculature in cancer and inflammation: New opportunities for in vivo imaging and drug delivery. J Controlled Release 172(2): 550-557.

Randolph GJ and Miller NE. (2014) Lymphatic transport of high-density lipoproteins and chylomicrons. J Clin Invest. 124(3): 929-935.

Rodrigues SF and Granger DN. (2015) Blood cells and endothelial barrier function. Tissue Barriers 3(1-2): e978720.

Rohner NA and Thomas SN. (2016) Melanoma growth effects on molecular clearance from tumors and biodistribution into systemic tissues versus draining lymph nodes. J Controlled Release 223: 99-108.

Roozendaal R, Mebius RE and Kraal G. (2008) The conduit system of the lymph node. Int Immunol. 20(12): 1483-1487.

Ruddle NH. (2016) High endothelial venules and lymphatic vessels in tertiary lymphoid organs: Characteristics, functions, and regulation. Front Immunol. 7: 491.

Schmid D, Park CG, Hartl CA, Subedi N, Cartwright AN, Puerto RB, Zheng Y, Maiarana J, Freeman GJ, Wucherpfennig KW, Irvine DJ and Goldberg MS. (2017) T cell-targeting nanoparticles focus delivery of immunotherapy to improve antitumor immunity. Nat Commun. 8(1): 1747.

Schudel A, Chapman AP, Yau MK, Higginson CJ, Francis D M, Manspeaker MP, Avecilla ARC, Rohner NA, Finn MG and Thomas SN. (2020) Programmable multistage drug delivery to lymph nodes. Nature Nanotechnol. 15(6): 491-499.

Schudel A, Francis DM and Thomas SN. (2019) Material design for lymph node drug delivery. Nature Rev Materials 4(6): 415-428.

Shang T, Liang J, Kapron CM and Liu J. (2019) Pathophysiology of aged lymphatic vessels. Aging 11(16): 6602-6613.

Spurrell EL and Lockley M. (2014) Adaptive immunity in cancer immunology and therapeutics. Ecancermedicalscience 8: 441.

Su JL, Yen CJ, Chen PS, Chuang SE, Hong CC, Kuo IH, Chen HY, Hung MC and Kuo ML. (2007) The role of the VEGF-C/VEGFR-3 axis in cancer progression. British J Cancer 96(4): 541–545.

Swartz MA and Lund AW. (2012) Lymphatic and interstitial flow in the tumour microenvironment: linking mechanobiology with immunity. Nature Rev Cancer 12(3): 210-219.

Takeda K, Mori S and Kodama T. (2017) Study of fluid dynamics reveals direct communications between lymphatic vessels and venous blood vessels at lymph nodes of mice. J Immunol Methods 445: 1-9.

Tamburini BA, Burchill MA and Kedl RM. (2014) Antigen capture and archiving by lymphatic endothelial cells following vaccination or viral infection. Nat Commun. 5(1): 3989.

Teh YC, Ding JL, Ng LG and Chong SZ. (2019) Capturing the fantastic voyage of monocytes through time and space. Front Immunol. 10: 834.

Thomas OS and Weber W. (2019) Overcoming physiological barriers to nanoparticle delivery—Are we there yet? Front Bioeng Biotechnol. 7: 415.

Thomas SN, Rohner NA and Edwards EE. (2016) Implications of lymphatic transport to lymph nodes in immunity and immunotherapy. Annual Rev Biomed Engineer. 18(1): 207-233.

Thomas SN, Rutkowski JM, Pasquier M, Kuan EL, Alitalo K, Randolph GJ and Swartz MA. (2012) Impaired humoral immunity and tolerance in *K14-VEGFR-3-Ig* mice that lack dermal lymphatic drainage. J Immunol. 189(5): 2181-2190.

Toy R, Hayden E, Shoup C, Baskaran H and Karathanasis E. (2011) The effects of particle size, density and shape on margination of nanoparticles in microcirculation. Nanotechnology 22(11): 115101.

Trac N, Chen LY, Zhang A, Liao CP, Poon C, Wang J, Ando Y, Joo J, Garri C, Shen K, Kani K, Gross ME and Chung EJ. (2021) CCR2-targeted micelles for anti-cancer peptide delivery and immune stimulation. J Controlled Release 329: 614-623.

Trevaskis NL, Charman WN and Porter CJH. (2008) Lipid-based delivery systems and intestinal lymphatic drug transport: A mechanistic update. Adv Drug Deliv Rev. 60(6): 702-716.

Trevaskis NL, Kaminskas LM and Porter CJH. (2015) From sewer to saviour — targeting the lymphatic system to promote drug exposure and activity. Nature Rev Drug Discovery 14(11): 781-803.

Tseng YC, Xu Z, Guley K, Yuan H and Huang L. (2014) Lipid–calcium phosphate nanoparticles for delivery to the lymphatic system and SPECT/CT imaging of lymph node metastases. Biomaterials 35(16): 4688-4698.

Uhl M, Aulwurm S, Wischhusen J, Weiler M, Ma JY, Almirez R, Mangadu R, Liu YW, Platten M, Herrlinger U, Murphy A, Wong DH, Wick W, Higgins LS and Weller M. (2004) SD-208, a novel transforming growth factor β receptor i kinase inhibitor, inhibits growth and invasiveness and enhances immunogenicity of murine and human glioma cells ***in vitro*** and ***in vivo***. Cancer Res. 64(21): 7954-7961.

Ulintz PJ, Greenson JK, Wu R, Fearon ER and Hardiman KM. (2018) Lymph node metastases in colon cancer are polyclonal. Clin Cancer Res. 24(9): 2214-2224.

Vu MN, Rajasekhar P, Poole DP, Khor SY, Truong NP, Nowell CJ, Quinn JF, Whittaker M, Veldhuis NA and Davis TP. (2019) Rapid assessment of nanoparticle extravasation in a microfluidic tumor model. ACS Appl Nano Mater. 2(4): 1844-1856.

Wang J, Masehi-Lano JJ and Chung EJ. (2017) Peptide and antibody ligands for renal targeting: nanomedicine strategies for kidney disease. Biomaterials Sci. 5(8): 1450-1459.

Wang L and Xia ZJ. (2013) Combination of albumin-bound paclitaxel and pegylated liposomal doxorubicin is effective in treatment of heavily treated relapsed/refractory diffuse large B-cell lymphoma: a case report. Leukemia Lymphoma 54(11): 2553-2555.

Wang P, Zhao P, Dong S, Xu T, He X and Chen M. (2018) An albumin-binding polypeptide both targets cytotoxic T lymphocyte vaccines to lymph nodes and boosts vaccine presentation by dendritic cells. Theranostics 8(1): 223-236.

Wennhold K, Shimabukuro-Vornhagen A and von Bergwelt-Baildon M. (2019) B cell-based cancer immunotherapy. Transfusion Med Hemotherapy 46(1): 36-46.

Willard-Mack CL. (2006). Normal structure, function, and histology of lymph nodes. Toxicol Pathol. 34(5): 409-424.

Witmer AN, van Blijswijk BC, Dai J, Hofman P, Partanen TA, Vrensen GFJM and Schlingemann RO. (2001) VEGFR-3 in adult angiogenesis. J Pathol. 195(4): 490-497.

Xiao K, Li Y, Luo J, Lee JS, Xiao W, Gonik AM, Agarwal RG and Lam KS. (2011) The effect of surface charge on in vivo biodistribution of PEG-oligocholic acid based micellar nanoparticles. Biomaterials, 32(13): 3435-3446

Yáñez JA, Wang SWJ, Knemeyer IW, Wirth MA and Alton KB. (2011) Intestinal lymphatic transport for drug delivery. Adv Drug Deliv Rev. 63(10–11): 923-942.

Yao L, Xue X, Yu P, Ni Y and Chen F. (2018) Evans blue dye: A revisit of its applications in biomedicine. Contrast Media Molec Imaging 2018: 1-10.

Yeh YW, Cheng CC, Yang ST, Tseng CF, Chang TY, Tsai SY, Fu E, Chiang CP, Liao LC, Tsai PW, Yu YL and Su JL. (2017) Targeting the VEGF-C/VEGFR3 axis suppresses Slug-mediated cancer metastasis and stemness via inhibition of KRAS/YAP1 signaling. Oncotarget 8(3): 5603-5618.

Yohan D, Cruje C, Lu X and Chithrani DB. (2016) Size-dependent gold nanoparticle interaction at nano–micro interface using both monolayer and multilayer (Tissue-Like) cell models. Nanomicro Lett. 8(1): 44-53.

Yu X, Zhu W, Di Y, Gu J, Guo Z, Li H, Fu D and Jin C. (2017) Triple-functional albumin-based nanoparticles for combined chemotherapy and photodynamic therapy of pancreatic cancer with lymphatic metastases. Int J Nanomed. 12: 6771-6785.

Yuan Z, Shang Z, Gu J and He L. (2019) Renal targeting delivery systems. Future Med Chem. 11(17): 2237-2240.